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Acidic chitinase primes the protective immune response to gastrointestinal nematodes.

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Acidic chitinase primes the protective immune response to gastrointestinal nematodes

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Acidic mammalian chitinase (AMCase) is induced by allergens and helminths, yet its role in immunity is unclear. Here, using AMCase-deficient mice, we show that while AMCase deficiency reduced the number of type 2 innate lymphoid cells during allergen challenge, the enzyme was not required for establishment of type 2 inflammation in the lung in response to allergens or helminths. In contrast, AMCase-deficient mice displayed a profound defect in type 2 immunity following infection with the chitin-containing gastrointestinal nematodes *Nippostrongylus brasiliensis* and *Heligmosomoides polygyrus bakeri*. The impaired immunity was associated with reduced mucus production and decreased *Il13, Chil3, Retnlb*, and *Clca1* mRNA expression in the intestine. $CD103^+$ dendritic cells, which regulate T cell homing, were also reduced in mesenteric lymph nodes of infected AMCase-deficient mice. Thus, AMCase functions as a critical initiator of protective type 2 responses to intestinal nematodes, but is largely dispensable for allergic responses in the lung.

Chitin is the second most abundant polymer in nature, found as a structural component in fungi[1], arthropods[2], and parasitic nematodes[3, 4]. Mammals do not synthesize chitin, but they express two known enzymes that digest chitin: acidic mammalian chitinase (AMCase)[5] and chitotriosidase[6]. AMCase is expressed in the lung and the gastrointestinal tract of humans and mice, and its activity is markedly increased in epithelial cells and macrophages in response to the type 2 cytokines IL-4 and IL-13, yet its role in type 2 inflammation and immunity remains unclear[8, 9, 10]. Most studies investigating AMCase function have focused on its role in allergic lung disease. Mice congenitally lacking AMCase (AMCase-deficient) demonstrated little to no role for the enzyme in acute models of house-dust mite or ovalbumin (OVA)-induced allergy in the lung[11].

These findings contrasted with another published study where AMCase activity was neutralized with allosamidin or with a monoclonal antibody and reported marked diminution of IL-13-driven allergic inflammation, suggesting the enzyme might represent an attractive therapeutic target in allergic asthma[8]. Still, additional reports have proposed a protective role for AMCase. One showed the type 2 inflammatory response following chitin challenge was ameliorated in mice overexpressing AMCase[9] and another observed increased allergic lung disease in mice specifically lacking AMCase enzymatic activity[12]. The contrasting functional implications of AMCase highlighted in these studies have yet to be fully reconciled. Further, allergic inflammation recapitulates the prototypic type 2 response seen after helminth infection[13], but surprisingly, despite the discovery that AMCase is highly expressed after exposure to helminths[14], it has remained unknown if the enzyme plays any role in the host immune response to these important human pathogens. In this study, we employed AMCase-deficient mice to dissect the role of the enzyme in several models of helminth infection and type 2 cytokine-driven airway inflammation. We show that although AMCase activity is largely dispensable for the development of allergic airway disease, the enzyme plays a critical role in the development of type 2 immunity to the gastrointestinal nematodes *Nippostrongylus brasiliensis* and *Heligmosomoides polygyrus bakeri*.

Results

Fewer ILCs but normal allergy in AMCase-deficient mice

Our initial studies were focused on reconciling the conflicting observations generated in models of allergic lung inflammation. We postulated that the higher doses of allergen used in a

previously published study (intranasal sensitization and challenges with 100μg house dust mite allergen, HDM) could have masked a role for AMCase, thus accounting for the different observations described above[11, 12]. Accordingly, we administered a low dose time-course (intranasal sensitization and challenges with 25μg and 5μg HDM, respectively) to AMCase-deficient mice. A day after the last of four challenge doses, we found the low doses of allergen increased the lung tissue expression of *Chia1*, the gene encoding AMCase in wild type mice (**Fig. 1a**), but that both wild type and AMCase-deficient mice exhibited similar pulmonary inflammatory pathology (**Fig. 1b**). In the tissue, AMCase abrogation had no significant impact on leukocyte or eosinophil accumulation or gene expression of the type 2 cytokines IL-5 and IL-13 (**Fig. 1c**). Also at this time-point, genes for type 2 initiators IL-33 and TSLP and for the alternative activation markers Relmα and Mrc1 were expressed at similar levels. Moreover, AMCase deficiency did not alter type 2 inflammation in the airways (**Fig. 1d**). To confirm these observations were not unique to HDM, we found similar results with papain, a non-chitinous allergen (**Supplementary Fig. 1**). We also extended our studies with a chronic model of HDM-induced allergy over 6 weeks, and here too, little to no role was revealed for AMCase (**Fig. 2**). These data bolster the conclusions of previous studies showing AMCase ablation does not have a significant effect on allergic airway pathology. They also support other reports that chitotriosidase is the primary active chitinase in the lung [7, 15]. We were able to detect gene expression of chitotriosidase in naive and allergic lung tissue although it was not elevated during the allergic response (**Fig. 1e**). Inquiries into whether chitotriosidase has a critical function in lung allergy and into the differences in mice with enzymatically-deficient AMCase and mice deficient in the entire AMCase protein remain to be answered.

Although AMCase ablation had no effect on the development of allergic disease, we found evidence that the innate type 2 response was reduced in AMCase-deficient mice. Following sensitization with HDM, fewer total leukocytes and fewer IL-5^{+}IL-13^{+} type 2 innate lymphoid cells (ILC2 cells) were observed in the lungs of AMCase-deficient mice (**Fig. 1f**). In addition, fewer ILCs expressed GATA-3 protein, a transcription factor critically required for the development of ILC2s[16]. Although AMCase-deficient mice ultimately overcame this defect at later time points, for the first time, these data suggest an important role for AMCase in type 2 immune priming upstream of ILCs. Whether this early immune priming defect explains why polymorphisms of AMCase are associated with airway allergy requires further investigation[17]. Our data indicate AMCase plays a role in type 2 immune initiation, but that the protein is not required for establishment of type 2 allergic inflammation in the lung.

Normal lung granuloma formation in AMCase-deficient mice

Next, we investigated the question of whether AMCase is required for the type 2 response to helminth parasites known to increase AMCase expression[14, 18]. We began by using a *Schistosoma mansoni* parasite egg-induced pulmonary granuloma model, where intravenously injected eggs that get trapped in the pulmonary vasculature trigger endothelial cell damage, potent type 2 inflammation, and IL-4- plus IL-13-dependent granuloma formation. *S. mansoni* egg exposure led to an IL-13-dependent upregulation of *Chia1*, which was more robust than that induced by HDM (**Fig. 3a**). Immunofluorescence staining localized the protein expression predominantly to bronchial epithelial cells (**Fig. 3b**). Despite the marked induction of AMCase in wild type lungs, granuloma formation (**Fig. 3c**), fibrosis (**Fig. 3d**), mucus production (**Fig. 3e**), granulomatous eosinophil accumulation (**Fig. 3f**), and total leukocyte accumulation in

bronchoalveolar lavage fluid (BALF) (**Fig. 3g**) were unimpaired in AMCase-deficient mice, demonstrating AMCase is not categorically required to mount wild type anti-parasite responses. Likewise, intratracheal delivery of schistosome egg antigen (SEA) upregulated *Chia1* in the lung, but the type 2 response was unaffected in AMCase-deficient mice, suggesting the lack of phenotype was not due to the route of antigen delivery (i.e. intravascular vs. epithelial exposure, **Supplementary Fig. 2**). Instead, the findings support the lung allergy studies that concluded AMCase does not exhibit important regulatory activity in the lung.

AMCase is critical for protection against *N. brasiliensis*

AMCase is highly expressed in the gastrointestinal tract (GI), but it was unknown whether AMCase is important for the development of immunity to gastrointestinal parasites that elicit a type 2-polarized protective immune response. GI roundworms or nematodes, which unlike the flatworm trematode *S. mansoni*, are known to contain chitin in their mouthparts, larval sheaths, and eggshells[3, 4], infect over 2 billion people, contributing to significant morbidity and mortality worldwide[19]. We sought to understand the role of AMCase in host protection using the rodent nematode, *N. brasiliensis*, which has been used extensively to study and model the immunobiology of human hookworm infestation[20]. In the murine model, after subcutaneous injection of infectious larvae, *N. brasiliensis* traverses the lung within 1-2 days before migrating up the trachea and entering the GI tract where larvae mature into egg-laying adults. Wild type mice successfully expel the worms by around 10-14 days after initial infection[21]. We enumerated the number of *N. brasiliensis* larvae in the wild type or AMCase-deficient guts 5, 8, 10, and 14 days post-infection. The number of worms that traversed the lungs and took residence in the gut on day 5 was not different between AMCase-sufficient and deficient mice (**Fig. 4a**).

Although wild type mice nearly completely expelled the worms by day 10 as expected, AMCase-deficient mice harbored significantly more worms in the intestine and most mice did not fully clear the infection even by day 14 (**Fig. 4a**). The significantly impaired host response resulting from AMCase deficiency was also characterized by a marked increase in the number of parasite eggs in the feces of infected AMCase-deficient mice (**Fig. 4b**).

To investigate the reasons for the impairment in host defense, we harvested lung, stomach, and intestinal tissue 8 days post-infection, the peak of expulsion in wild type mice. In the lung, *Chia1* expression was upregulated as previously described[9], but *Il13* and the majority of known effector molecules tested were expressed comparably between wild type and AMCase-deficient mice (**Supplementary Fig. 3**). Only *Chil3* (the gene encoding the chitinase-like protein, Ym1) expression was significantly impaired in AMCase-deficient lungs even a few days after worm passage—this is notable because Ym1 induces IL-17 and neutrophilic inflammation in the lung that was postulated to compromise the fitness of *N. brasiliensis* larvae[22]. Like the original description of AMCase[5], *Chia1* expression in the intestines was undetectable, but it was higher by at least one order of magnitude in the stomach than in the lung (**Fig. 4c**). In contrast to the lung where expression held steady, chitotriosidase gene expression was greatly diminished in intestines of AMCase-deficient mice during *N. brasiliensis* infection (**Fig. 4d**). Gene expression profiling in the intestine also correlated with a broadly impaired host response to *N. brasiliensis,* with AMCase-deficient mice exhibiting markedly reduced expression of *Il13* and several key downstream type 2 effector genes (**Fig. 4d**). *Il13* expression was reduced by more than 50%, and *Chil3* expression, upregulated over 2000-fold in infected wild type intestine, was nearly completely abrogated, approaching the levels found in uninfected mice. Perhaps most notably, AMCase was necessary for normal *Retnlb* expression, the gene encoding another mediator that

has previously been shown to be essential for normal nematode expulsion[21]. Expression of *Clca1*, the gene encoding a chloride channel (Gob5) involved in mucus production[23], was also reduced. This defect likely explains the diminished mucus production from intestinal goblet cells, which is also critical to the development of protective immunity[24] (**Fig. 4e**). Accordingly, the kinetics of *N. brasiliensis* clearance in the AMCase-deficient mice were similar to past studies done in mice deficient in IL-13 signaling[25, 26]. Collectively, our data show that AMCase is necessary for mice to mount normal type 2 immunity against *N. brasiliensis*.

AMCase ablation impairs type 2 immunity against *H.p. bakeri*

Lastly, because AMCase is expressed in the lung, we sought to explore if the defective type 2 response in AMCase-deficient mice following *N. brasiliensis* infection was also observed following primary and secondary infection with *H polygyrus bakeri*—a rodent nematode that is acquired orally, restricted to the GI tract, and does not migrate through the lungs. Also, in contrast to the *N. brasiliensis* model, wild type mice do not clear primary infection with *H. p. bakeri,* but upon antihelminthic treatment, subsequent infections are successfully eliminated – therefore, it is an ideal model to explore the role of AMCase in the development and maintenance of secondary immunity. There was a marked increase in *Chia1* mRNA expression in the stomach following infection that was absent in AMCase-deficient mice (**Fig. 5a**). Moreover, as expected, there was no difference in worm recoveries between the two groups of mice after a primary infection, but impairment of the host immune-mediated worm expulsion in AMCase-deficient mice was strikingly apparent after a secondary infection (**Fig. 5b**). Wild type mice had nearly cleared all adult *H. p. bakeri* worms 15 days after re-infection, but AMCase-deficient mice still harbored an average of ~50 worms. Even though the worm burden was not

affected by AMCase deficiency during primary infection, the fecundity of the worms differed significantly— *H. p. bakeri* egg output in the AMCase-deficient mice was more than three-fold greater than in wild type animals (**Fig. 5c**). We observed higher ATP uptake in the worms recovered from AMCase-deficient mice, correlating with the increased fecundity and suggesting increased worm vitality (**Fig. 5d**). As with *N. brasiliensis* infection, AMCase-deficient intestines expressed much less *Il13* after both primary and secondary *H. p. bakeri* infections (**Fig. 5e**). This again corresponded with significantly less *Chil3* and *Retnlb* expression in both infections. *Clca1* expression was significantly lower in AMCase-deficient mice, mirrored by less luminal and cellular mucus in the intestines (**Fig. 5f**). We ruled out that the immune defect is T cell intrinsic by transferring $CD4^+$ T cells from *H.p. bakeri*-infected wild type and AMCase-deficient mice into *H.p. bakeri*-infected TCRα-deficient mice. Recipients of cells from both cohorts were equally competent at clearing a primary infection (**Supplementary Fig. 4**).

Since we found evidence of defective immune priming in AMCase-deficient lungs (**Fig 1f**), we hypothesized that immune priming was also deficient in response to GI nematodes. In the duodenum, the type 2 alarmin *Il33* was expressed at similar levels in wild type and AMCase-deficient mice in the hours after *H. p. bakeri* worms first reached the proximal intestine (**Supplementary Fig. 5**). Amongst the leukocytes in the mesenteric lymph node at this time, however, we found the percentage and total number of $CD103^+$ $MHCII^+$ $CD11b^+$ dendritic cells was significantly diminished (**Fig. 5g**). $CD103^+$ DCs have been reported to have a unique capacity to induce gut-homing activity in responding T cells in the mesenteric lymph node[27]. These data show AMCase has a critical role in initiating type 2 immunity against *H.p. bakeri* in the GI tract of the host.

Discussion

Collectively, our data indicate AMCase regulates the early priming of type 2 immune responses in the lung and the GI tract, but that its role in generating protective anti-nematode immunity in the GI tract is much more critical than its role in the lung. AMCase-deficient mice and wild type mice develop similar acute and chronic type 2-driven allergic lung pathology in response to HDM, SEA, or papain inhalation. AMCase ablation also had no effect on the development of type 2-driven granuloma formation around helminth eggs in the lung. In contrast, AMCase is essential for optimal IL-13 production during infection with *N. brasiliensis* and *H. p. bakeri* infection, which is required to activate downstream anti-parasite effector molecules (e.g. Ym1, Relmβ), and mucus production that cooperatively facilitate parasite expulsion from the intestine.

It is possible that dominance of chitotriosidase in the lung might explain the different outcomes in the lung and gut; chitotriosidase gene expression was largely unaffected by AMCase ablation during allergen challenge but was greatly diminished in intestines of AMCase-deficient mice during *N. brasiliensis* infection. Also, because the stomach exhibits both constitutive and IL-13-inducible AMCase activity, this might serve as the critical location for the initiation of anti-nematode type 2 immunity as *N. brasiliensis* and *H. p. bakeri* both pass through the stomach on their way to the intestine. Indeed, a prominent role for AMCase in the stomach and duodenum is expected because its chitinolytic activity is greatest at low pH. AMCase functions as a true chitinase in the stomach as previously suggested[30]; therefore it is quickly transported into the duodenum where it may aid in the disruption, release, and/or processing of parasite-associated chitinous antigens that are critical for the initiation of protective type 2 responses in the gut. We found no evidence that the defective type 2 responses observed during nematode infection were

T cell intrinsic. Instead, it is likely that the absence of AMCase results in less processing of parasite chitin in the stomach, which would reduce the release of chitin fragments and other antigens that serve as adjuvants for type 2 immunity[9]. This is certainly consistent with the decrease in type 2 immunity and increase in parasite load, fecundity, and worm vitality observed in the infected AMCase-deficient mice. Finding fewer $CD103^+$ DCs in the intestine-draining mesenteric lymph nodes of AMCase-deficient mice also indicates AMCase stimulates the anti-parasite immune response. There is no existing literature about a required role for $CD103^+$ DCs in type 2 responses to parasites[28], but there is evidence they are the major migratory dendritic cell population that presents soluble luminal antigen to T cells[29].

AMCase has long been associated with type 2 cytokine responses that mediate pathogenic allergic inflammation and beneficial immunity to gastrointestinal parasites. The role of AMCase in these widespread diseases, however, remained unclear. We show here that AMCase has a critical and non-redundant role in type 2 immune priming during infection with the chitin-containing nematode parasites *N. brasiliensis and H.p. bakeri*. Because AMCase exhibited a less significant regulatory role in models of allergic lung inflammation and humans lack the machinery to synthesize chitin, global exposure to geohelminths rather than airborne allergens likely provided critical evolutionary pressure to preserve AMCase activity in the human genome.

Accession codes

Rplp2: NM_026020, *Chia1:* NM_023186, *Il4:* NM_021283, *Il13:* NM_008355, *Chil3:* NM_009892, *Relnlb:* NM_023881, *Retnla:* NM_020509, *Clca1:* NM_017474, *Il5:* NM_010558,

Il25: NM_080729, *Il33:* NM_001164724, *Tslp:* NM_021367, *Mrc1:* NM_008625, *Chit1:* NM_001284525.

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Author contributions

KMV TRR TAW conceived and designed the experiments; KMV ADS KMH LAB RWT SW JFU RQP JS performed the experiments; IM KB performed immunofluorescence techniques; KMV TRR ADS AWC LB LAB MMK TAW JFU RQP analyzed the data; ADS AWC IM JFU LJF contributed reagents; KMV TRR TAW wrote the paper.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Main Text References

1. Lenardon, M.D., Munro, C.A. & Gow, N.A. Chitin synthesis and fungal pathogenesis. *Curr Opin Microbiol* **13**, 416-423 (2010).

2. Neville, A.C., Parry, D.A. & Woodhead-Galloway, J. The chitin crystallite in arthropod cuticle. *J Cell Sci* **21**, 73-82 (1976).

3. Veronico, P. *et al.* Nematode chitin synthases: gene structure, expression and function in Caenorhabditis elegans and the plant parasitic nematode Meloidogyne artiellia. *Molecular genetics and genomics : MGG* **266**, 28-34 (2001).

4. Foster, J.M., Zhang, Y., Kumar, S. & Carlow, C.K. Parasitic nematodes have two distinct chitin synthases. *Molecular and biochemical parasitology* **142**, 126-132 (2005).

5. Boot, R.G. *et al.* Identification of a novel acidic mammalian chitinase distinct from chitotriosidase. *The Journal of biological chemistry* **276**, 6770-6778 (2001).

6. Renkema, G.H., Boot, R.G., Muijsers, A.O., Donker-Koopman, W.E. & Aerts, J.M. Purification and characterization of human chitotriosidase, a novel member of the chitinase family of proteins. *The Journal of biological chemistry* **270**, 2198-2202 (1995).

7. Wiesner, D.L. *et al.* Chitin recognition via chitotriosidase promotes pathologic type-2 helper T cell responses to cryptococcal infection. *PLoS pathogens* **11**, e1004701 (2015).

8. Zhu, Z. *et al.* Acidic mammalian chitinase in asthmatic Th2 inflammation and IL-13 pathway activation. *Science* **304**, 1678-1682 (2004).

9. Reese, T.A. *et al.* Chitin induces accumulation in tissue of innate immune cells associated with allergy. *Nature* **447**, 92-96 (2007).

10. Ramanathan, M., Jr., Lee, W.K. & Lane, A.P. Increased expression of acidic mammalian chitinase in chronic rhinosinusitis with nasal polyps. *American journal of rhinology* **20**, 330-335 (2006).

11. Fitz, L.J. *et al.* Acidic mammalian chitinase is not a critical target for allergic airway disease. *American journal of respiratory cell and molecular biology* **46**, 71-79 (2012).

12. Kim, L.K. *et al.* AMCase is a crucial regulator of type 2 immune responses to inhaled house dust mites. *Proc Natl Acad Sci U S A* **112**, E2891-2899 (2015).

13. Wynn, T.A. Type 2 cytokines: mechanisms and therapeutic strategies. *Nature reviews. Immunology* **15**, 271-282 (2015).

14. Nair, M.G. *et al.* Chitinase and Fizz family members are a generalized feature of nematode infection with selective upregulation of Ym1 and Fizz1 by antigen-presenting cells. *Infection and immunity* **73**, 385-394 (2005).

15. Seibold, M.A. *et al.* Chitotriosidase is the primary active chitinase in the human lung and is modulated by genotype and smoking habit. *J Allergy Clin Immunol* **122**, 944-950 e943 (2008).

16. Yagi, R. *et al.* The transcription factor GATA3 is critical for the development of all IL-7Ralpha-expressing innate lymphoid cells. *Immunity* **40**, 378-388 (2014).

17. Bierbaum, S. *et al.* Polymorphisms and haplotypes of acid mammalian chitinase are associated with bronchial asthma. *American journal of respiratory and critical care medicine* **172**, 1505-1509 (2005).

18. Sandler, N.G., Mentink-Kane, M.M., Cheever, A.W. & Wynn, T.A. Global gene expression profiles during acute pathogen-induced pulmonary inflammation reveal divergent roles for Th1 and Th2 responses in tissue repair. *J Immunol* **171**, 3655-3667 (2003).

19. Albonico, M. *et al.* Controlling soil-transmitted helminthiasis in pre-school-age children through preventive chemotherapy. *PLoS neglected tropical diseases* **2**, e126 (2008).

20. Zaph, C., Cooper, P.J. & Harris, N.L. Mucosal immune responses following intestinal nematode infection. *Parasite immunology* **36**, 439-452 (2014).

21. Herbert, D.R. *et al.* Intestinal epithelial cell secretion of RELM-beta protects against gastrointestinal worm infection. *J Exp Med* **206**, 2947-2957 (2009).

22. Sutherland, T.E. *et al.* Chitinase-like proteins promote IL-17-mediated neutrophilia in a tradeoff between nematode killing and host damage. *Nature immunology* **15**, 1116-1125 (2014).

23. Sabo-Attwood, T. *et al.* Gene expression profiles reveal increased mClca3 (Gob5) expression and mucin production in a murine model of asbestos-induced fibrogenesis. *The American journal of pathology* **167**, 1243-1256 (2005).

24. Hasnain, S.Z. *et al.* Muc5ac: a critical component mediating the rejection of enteric nematodes. *J Exp Med* **208**, 893-900 (2011).

25. McKenzie, G.J., Fallon, P.G., Emson, C.L., Grencis, R.K. & McKenzie, A.N. Simultaneous disruption of interleukin (IL)-4 and IL-13 defines individual roles in T helper cell type 2-mediated responses. *J Exp Med* **189**, 1565-1572 (1999).

26. Urban, J.F., Jr. *et al.* IL-13, IL-4Ralpha, and Stat6 are required for the expulsion of the gastrointestinal nematode parasite Nippostrongylus brasiliensis. *Immunity* **8**, 255-264 (1998).

27. Johansson-Lindbom, B. *et al.* Functional specialization of gut CD103+ dendritic cells in the regulation of tissue-selective T cell homing. *J Exp Med* **202**, 1063-1073 (2005).

28. Iwasaki, A. & Medzhitov, R. Control of adaptive immunity by the innate immune system. *Nature immunology* **16**, 343-353 (2015).

29. Jaensson, E. *et al.* Small intestinal CD103+ dendritic cells display unique functional properties that are conserved between mice and humans. *J Exp Med* **205**, 2139-2149 (2008).

30. Strobel, S., Roswag, A., Becker, N.I., Trenczek, T.E. & Encarnacao, J.A. Insectivorous bats digest chitin in the stomach using acidic mammalian chitinase. *PloS one* **8**, e72770 (2013).

Figure legends

Figure 1. AMCase-deficient mice develop acute HDM-induced lung allergy despite diminished ILC2s.

(**a**) Quantitative PCR analysis of AMCase gene (*Chia1*) expression in lung tissue from wild type mice or AMCase-deficient mice (AMCase-KO) sensitized and challenged intranasally with PBS (n = 9 mice per genotype) or house dust mite (HDM) (n =21 mice per genotype) expressed relative to PBS-treated wild type. (**b**) Lung pathology of mice in **a** scored using H&E (PBS: n = 3, HDM: n = 7) and Periodic acid-Schiff (PAS) (PBS: n = 6, HDM: n = 15; pooled from 2 experiments) stains (scale bars=100μm). (**c**) Lung tissue leukocyte (PBS: n = 6, HDM: n = 15 pooled from 2 experiments) and eosinophil quantification (PBS: n = 9, HDM: n = 22; pooled from 3 experiments) from three lung lobes of mice in **a** and gene expression analysis (PBS: n =

9, HDM: $n = 22$; pooled from 3 experiments). (**d**) Leukocyte and eosinophil quantification and intracellular cytokine analysis of lymphocytes collected from bronchoalveolar lavage fluid (BALF) of mice in **a** (PBS: $n = 9$, HDM: $n = 22$). (**e**) Chitotriosidase gene expression in lung tissue (representative of naive and allergic lungs) (wild type: $n = 3$, AMCase-KO: $n = 3$) shown relative to expression of the housekeeping gene, *Rplp2*. (**f**) Quantification of total lung leukocytes, IL-4^+IL-13^+ type 2 innate lymphoid cells (ILC2s), and Gata3^+ cells amongst ILCs from wild type or AMCase-KO mice intranasally sensitized with PBS ($n = 3$) or HDM ($n = 7$). *$P<0.05$, **$P<0.01$ (Student's *t*-test). Data are pooled from three experiments (**a,d**) or are from one experiment representative of three independent experiments with similar results (**e,f**). Error bars represent standard error of the mean, and each data point represents a value for an individual mouse.

Figure 2. Chronic HDM induces similar allergy in lungs of wild type and AMCase-deficient mice.

(**a**) Histopathology scoring and representative sections stained with H&E (scale bars=200μm) from wild type or AMCase-KO mice sensitized and challenged intranasally with PBS ($n = 3$ mice per genotype) or house dust mite (HDM) ($n = 8$ mice per genotype) for 45d. (**b**) Quantification of eosinophils in lung tissue of mice in **a** (PBS: $n = 3$, HDM: $n = 9$). (**c**) Intracellular cytokine analysis of lung tissue lymphocytes of mice in **a** (PBS: $n = 3$, HDM: $n = 9$). (**d**) Quantification of eosinophils in BALF of mice in **a** (PBS: $n = 3$, HDM: $n = 9$). (**e**) Intracellular cytokine analysis of BALF lymphocytes of mice in **a** (PBS: $n = 3$, HDM: $n = 9$). Data are pooled from two experiments with similar results. Error bars represent standard error of the mean, and each data point represents a value for an individual mouse.

Figure 3. AMCase-deficient mice develop *S. mansoni* egg-induced lung granulomas.

(**a**) Quantitative PCR analysis of *Chia1* expression in lung tissue from wild type mice (n = 5) or IL-13-deficient mice (IL-13-KO) (n = 5) seven days after intravenous injection of *S. mansoni* eggs. (**b**) Immunofluorescence of lung airway sections seven days after intravenous egg injection. (**c**) Four or seven days after intravenous egg injection into wild type (D4: n = 8, D7: n = 11) or AMCase-KO mice (D4: n = 11, D7: n = 10), granulomatous lung pathology was measured using Giemsa stain. (**d**) Lung sections from mice in **c** scored for fibrosis by Picrosirius stain. (**e**) Lung sections from mice in **c** scored for mucus in goblet cells by PAS stain. (**f**) Quantification of eosinophils comprising granulomas from Giemsa-stained lung sections of mice in **c**. (**g**) Quantification of BALF cells from mice in **c**. Scale bar=50 μm (**b**), 100 μm (**c,d,e**). Data are representative of two experiments with similar results. Error bars represent standard error of the mean, and each data point represents a value for an individual mouse.

Figure 4. AMCase is critical for type 2-mediated protection against *N. brasiliensis*.

(**a**) Quantification of nematodes from intestines of wild type or AMCase-KO mice (n = 5 per genotype per time-point) on days after infection. (**b**) Fecal egg counts (D6: n = 14 mice per genotype; D7: n = 14 per genotype; D8: n = 13 per genotype; D9: n = 8 per genotype; D10: n = 8 per genotype) (**c**) Quantitative PCR analysis of *Chia1* expression in tissues from naïve wild type mice (n = 3) shown relative to expression of the housekeeping gene, *Rplp2*., (**d**) Quantitative PCR analysis of gene expression in small intestinal tissue of wild-type mice (n = 7) or AMCase-KO mice (n = 7) 8 days post-*N.brasiliensis* infection shown relative to expression in naïve mice. (**e**) Representative PAS staining from small intestinal sections from mice in **d** (scale bar=100

μm). *P<0.05, **P<0.01, ***P<0.001 (Student's t-test). Data are representative of two experiments with similar results. Error bars represent standard error of the mean, and each data point represents a value for an individual mouse.

Figure 5. Type 2 immunity is impaired against *H. p. bakeri* in AMCase-deficient mice. (**a**) Quantitative PCR analysis of *Chia1* expression in stomach tissue from wild type mice (n = 7) or AMCase-KO mice (n = 6) 15 days after primary infection with *H. p. bakeri*. (**b**) Adult worm burden in the intestines 15 days after primary (1^o) or secondary (2^o) challenge of wild type mice (n = 8) or AMCase-KO mice (n = 7). (**c**) Fecal egg counts 12 days after primary or secondary challenge of wild type mice (n = 8) or AMCase-KO mice (n = 7). (**d**) Adult worm ATP levels 12 days post-primary challenge of wild type mice (n = 2) or AMCase-KO mice (n = 7). (**e**) Quantitative PCR analysis of gene expression in small intestinal tissue of wild-type mice (n = 7) or AMCase-KO mice (n = 7) 12 days post-infection shown relative to expression in naïve mice. (**f**) PAS staining of representative small intestinal tissue sections 12 days post-secondary challenge (scale bar=200μm; arrows indicate parasites). (**g**) Quantification of mesenteric lymph node dendritic cells from wild type mice (n = 8) or AMCase-KO mice (n = 7) 3.5 days post-infection (Inf) or from uninfected (UI) wild type or AMCase-KO (n = 3 per genotype). *P<0.05, **P<0.01, ***P<0.001 (Student's t-test). Data are representative of two experiments with similar results. Error bars represent standard error of the mean, and each data point represents a value for an individual mouse.

Online Methods

457 **Animals**

458 The National Institute of Allergy and Infectious Diseases Division of Intramural Research
459 Animal Care and Use Program, as part of the National Institutes of Health Intramural Research
460 Program, approved all of the experimental procedures (protocol "LPD 16E"). The Program
461 complies with all applicable provisions of the Animal Welfare Act
462 (http://www.aphis.usda.gov/animal_welfare/downloads/awa/awa.pdf) and other federal statutes
463 and regulations relating to animals. AMCase-deficient mice on a C57BL/6N background were
464 kindly provided by Lori Fitz and Pfizer. Wild type C57BL/6N mice, and IL-13-deficient and
465 TCRα-deficient mice on a C57BL/6N background, were obtained from Taconic Farms Inc. Male
466 and female mice between the ages of 6 weeks and 12 weeks were used randomly to begin
467 experimental models because of limited availability, and no sex-specific differences were
468 observed. Groups in individual experiments were sex-matched and age-matched. All animals
469 were housed under specific pathogen-free conditions at the National Institutes of Health in an
470 American Association for the Accreditation of Laboratory Animal Care-approved facility.
471

472 ***S. mansoni* egg-induced lung granuloma model**

473 5000 *S. mansoni* eggs (Biomedical Research Institute) were injected intraperitoneally on day 0 to
474 sensitize mice. On day 14, mice were challenged intravenously with 5000 live eggs containing
475 mature embryos again before lungs were harvested on day 18 or 21.
476

477 **Schistosome egg antigen-induced lung inflammation model**

478 Schistosome egg antigen (SEA) was obtained from sterile LPS-free liver-derived eggs from mice
479 chronically infected with *Schistosoma mansoni*. Mice were intratracheally sensitized and

challenged with 10 μg SEA on days 0, 7, 14, 16, and 18. SEA was administered in 30 μl saline to mice anesthetized with isoflurane. Lungs were lavaged and harvested for analysis on day 19.

Acute house dust mite allergen-induced lung inflammation model

Mice were intranasally sensitized with 25 μg of house dust mite (HDM, Greer) or PBS on days 0, 1, and 2. On days 15, 16, 17, and 18, mice were intranasally challenged with 5 μg of HDM or PBS. HDM was administered in 30 μl PBS to mice anesthetized with isoflurane. Lungs were lavaged and harvested for analysis on day 19.

Chronic house dust mite allergen-induced lung inflammation model

Mice were intranasally sensitized with 25 μg of house dust mite (HDM, Greer) or PBS on days 0, 1, and 2. On days 14, 15, 16, 28, 29, 30, 42, 43, and 44, mice were intranasally challenged with 5 μg of HDM or PBS. Lungs were lavaged and harvested for analysis on day 45.

Acute papain-induced lung inflammation model

Mice were intranasally sensitized with 12.5 μg of papain (*Carica papaya*, EMD Millipore) or water on days 0, 1, and 2. On days 15, 16, 17, and 18, mice were intranasally challenged with 10 μg of papain or PBS. Papain was administered in 30 μl PBS to mice anesthetized with isoflurane. Lungs were lavaged and harvested for analysis on day 19.

***Nippostrongylus brasiliensis* infection**

Third-stage (L3) larvae were prepared as described previously[31], and 500 were injected subcutaneously into the nape of the neck of each mouse. Feces were collected on days 6-10

post-infection for egg counts. Adult worms were counted in the small intestine on days 5, 8, 10, or 14. Representative sections of lung and small intestinal tissue were taken for histology and qPCR analysis on day 8.

***Heligmosomoides polygyrus bakeri* infection**

As described previously[32], mice were inoculated periorally with 200 L3, and 2 weeks later, worms were expelled by administering 1-2 mg pyrantal pamoate. 4 weeks later, the sensitized mice were challenged with *H. p. bakeri* (secondary) while naive mice were inoculated with 200 L3 for the first time as controls (primary). 12 days after this, mice were sacrificed for analysis. Eggs were counted in the feces, and tissue was collected for histology and gene expression assay by qPCR. Adult worms were counted in the intestines 15 days after primary inoculation and secondary challenge. The ATPLite Luminescence Assay System (Perkin Elmer) was used to measure ATP content of adult worms collected from the intestines of primary infected mice. For some experiments, the proximal small intestine and mesenteric lymph nodes were harvested 3.5 days post-infection for studies of the innate immune response.

T cell transfer

Wild type and AMCase-deficient mice were subjected to primary *H. p. bakeri* infection with 200 L3. Two weeks later, mesenteric lymph nodes were harvested and ground into a single cell suspension through a 70 μm filter on ice. $CD4^+$ T cells were isolated from the leukocytes using Dynabeads Untouched Mouse CD4 Cells kit (Invitrogen). TCRα-deficient mice were infected with *H. p. bakeri* and on the same day were injected intravenously with $2x10^5$ T cells from wild

type mice, $2x10^5$ T cells from AMCase-deficient mice, or no T cells. *H.p. bakeri* adult worms were counted from the recipient mice 15 days later.

Bronchoalveolar lavage

1 ml of ice-cold PBS supplemented with 5 mM EDTA was injected through the trachea into the lungs and aspirated using a syringe.

Histopathology

Representative samples of lung or intestinal tissue were fixed in Bouin-Hollande solution, embedded in paraffin for sectioning, and stained (Histopath of America) with Wright's Giemsa to measure eosinophil accumulation, hematoxylin and eosin to analyze inflammation, Picrosirius red to analyze fibrosis, or Periodic acid-Schiff (PAS) stain for analysis of mucus production. A blinded pathologist measured the diameter of approximately 30 granulomas and quantified granulomatous eosinophils in Giemsa-stained sections of each sample with granulomatous pathology. The blinded pathologist also scored the stained sections for fibrosis and mucus production. Images were scanned at 40X with an Aperio ScanScope (Leica Biosystems).

Immunofluorescence staining and microscopy

Immunodetection of AMCase was performed on lung and intestinal tissue sections with rabbit sera immunized against recombinant murine AMCase that was a gift from MedImmune. MedImmune validated the antisera in-house for use in mice.

Leukocyte isolation from lung tissue for analysis by flow cytometry

About 200 mg of lung tissue was ground into a single-cell suspension through a 100-µm nylon mesh. Leukocytes were separated on a 40% Percoll (Sigma-Aldrich) gradient (2000 rpm for 15 min) and treated for 2 min with 1 ml ACK (ammonium chloride–potassium bicarbonate) lysis buffer to lyse erythrocytes. After 3 hours of stimulation with phorbol 12-myristate13-acetate (PMA, 10 ng/ml), ionomycin (1 µg/ml), and Brefeldin A (BFA, 10 µg/ml), leukocytes were fixed and permeabilized for 30 minutes (Cytofix/Cytoperm buffer; BD Biosciences) and then stained for 30 minutes with antibodies for CD4 (1:200 dilution; RM4-5; eBioscience), IL-4 (1:200 dilution; 11B11; eBioscience), IL-5 (1:200 dilution; TRFK5; BD Pharmingen), and IL-13 (1:200 dilution; eBio13A; eBioscience) diluted in the Permwash buffer (BD Biosciences). Unstimulated lung leukocyte aliquots were set aside and stained for 30 minutes with anti-SiglecF (1:200 dilution; E50-2440; BD Biosciences). Positive SiglecF staining and scatter profiling were used to identify eosinophils by flow cytometry. Expression of CD4, SiglecF, and the intracellular cytokines was analyzed with a BD FACSCanto II flow cytometer and FlowJo v.7.6 software (Tree Star). For some experiments, leukocytes were isolated as above approximately 12 hours after the last of three sensitization doses of house dust mite. Some cells were stimulated as described above, washed with Hank's Balanced Salt Solution, and stained with Live/Dead Fixable Blue (Life Technologies), anti-CD16/32 (1:500 dilution; Biolegend), and a biotin-conjugated lineage cocktail (1:100 dilution; eBioscience) composed of antibodies against CD8 (eBioH35-17.2), CD11b (M1/70), CD19 (MB19-1), CD49b (DX5), Gr-1 (RB6-8C5), NK1.1 (PK136), γδTCR (eBioGL3), TER-119, and CD11c (N418) for 20 minutes at 4^{o}C. Next, cells were washed with FACS buffer and stained with a streptavidin-conjugated antibody and antibodies against CD16/32, Thy1.2 (1:400 dilution; 53-2.1; Biolegend), CD45 (1:400 dilution; 30-F11; Biolegend), and TCRβ (1:200 dilution; H57-597; eBioscience) for 30 minutes at 4^{o}C.

The cells were washed again with FACS buffer before being fixed with 2% paraformaldehyde for 15 minutes at room temperature. Cells were then permeabilized by washing with 0.5% saponin (Sigma) and stained with antibodies for CD4 (1:500 dilution; RM4-5; BDBioscience), IL5 (1:200 dilution), IL13 (1:100 dilution), and CD16/32 (1:500 dilution) in the same buffer for 45 minutes at 4^{o}C. The cells were again washed in 0.5% saponin before being resuspended in FACS buffer for analysis with a BD LSRFortessa flow cytometer. Type 2 innate lymphoid cells were identified as live Lin^{-} TCRβ^{-} CD4^{-} Thy1.2^{+} CD45^{+} IL-5^{+} IL-13^{+}. Some cells were left unstimulated for measurement of Gata3 expression. These cells were processed as above until they were permeabilized with Fixation/Permeabilization solution (eBioscience) and then stained with antibodies against CD4 (1:400 dilution; RM4-5; Biolegend) and Gata3 (1:40 dilution; L50-823; BDBiosciences) and washed with permeabilization buffer (eBioscience). Gata3^{+} innate lymphoid cells were also identified with a BD LSRFortessa. All antibodies are commercially available, and validation profiles and references are available on corresponding commercial websites.

Leukocyte isolation from mesenteric lymph nodes for dendritic cell identification

3.5 days post-infection with *H.p. bakeri*, mesenteric lymph nodes were ground into a single-cell suspension through a 70-μm cell strainer on ice. Leukocytes were fixed and then stained for 30 minutes with antibodies for CD16/32 (1:100 dilution; BDBiosciences), CD45 (1:200 dilution; 30-F11; Biolegend), Ly6G (1:400 dilution; 1A8; BD Pharmingen), CD11c (1:200 dilution; 3.9; Biolegend), MHCII 1Ab (1:200 dilution; M5/114.15.2; eBioscience), CD11b (1:200 dilution; M1/70; Biolegend), and CD103 (1:200 dilution; M290; BDBiosciences). CD45^{+} Ly6G^{-} CD11c^{+} MHCII^{+} CD11b^{+} CD103^{+} cells were identified with a BD LSRFortessa and FlowJo v.7.6

software. All antibodies are commercially available, and validation profiles and references are available on corresponding commercial websites.

RNA isolation and quantitative real-time PCR

Lung, stomach, or intestinal tissue was homogenized in TRIzol Reagent (Life Technologies) using Precellys 24 (Bertin Technologies). Total RNA was extracted from the homogenate by addition of chloroform followed by the recommendations of the MagMax-96 Total RNA Isolation Kit (Life Technologies). RNA was then reverse transcribed using SuperScript II Reverse Transcriptase (Life Technologies). Real-time RT-PCR was performed on an ABI Prism 7900HT Sequence Detection System (Applied Biosystems). Quantities of mRNA expressed by a particular gene were determined using Power SYBR Green PCR Master Mix (Applied Biosystems), normalized to ribosomal protein, large, P2 (RPLP2) mRNA levels in each sample, and then articulated as a relative increase or decrease compared with mRNA levels expressed by the same gene in naive controls. We used the following primer sequences: *Rplp2:* forward – *5'- TACGTCGCCTCTTACCTGCT-3'*, reverse-*5'- GACCTTGTTGAGCCGATCAT-3'*; *Chia1:* forward -*5'- TGGACCTGGACTGGGAATACC-3'*, reverse-*5'- TGGGCCTGTTGCTCTCAATAG-3'*; *Il4:* forward -*5'- ACGAGGTCACAGGAGAAGGGA-3'*, reverse-*5'- AGCCCTACAGACGAGCTCACTC-3'*; *Il13:* forward -*5'- CCTCTGACCCTTAAGGAGCTTAT-3'*, reverse-*5'- CGTTGCACAGGGGAGTCT-3'*; *Chil3:* forward -*5'- CATGAGCAAGACTTGCGTGAC-3'*, reverse-*5'- GGTCCAAACTTCCATCCTCCA-3'*; *Relnlb:* forward -*5'- CGTCTCCCTTTTCCCACTG-3'*, reverse-*5'- CAGGAGATCGTCTTAGGCTCTT-3'*; *Retnla:* forward -*5'-CCCTCCACTGTAACGAAGACTC-3'*, reverse-*5'- CACACCCAGTAGCAGTCATCC-3'*; *Clca1:* forward -*5'- AGGAAAACCCCAAGCAGTG-3'*,

reverse-*5'- GCACCGACGAACTTGATTTT-3'*; *Il5:* forward – *5'-*
TGACAAGCAATGAGACGATGAGG -3', reverse-*5'- ACCCCCACGGACAGTTTGATTC -3'*;
Il33: forward – *5'- CACATTGAGCATCCAAGGAA -3'*, reverse-*5'-*
AACAGATTGGTCATTGTATGTACTCAG -3'; *Tslp:* forward – *5'-*
ACGGATGGGGCTAACTTACAA -3', reverse-*5'- AGTCCTCGATTTGCTCGAACT -3'*; *Il25:*
forward – *5'- ACAGGGACTTGAATCGGGTC -3'*, reverse-*5'- TGGTAAAGTGGGACGGAGTTG*
-3'; *Mrc1:* forward – *5'- CCCAAGGGCTCTTCTAAAGCA*
-3', reverse-*5'- CGCCGGCACCTATCACA -3'*; *Chit1:* forward – *5'- TGGGCAGGTGTGATGACTCT*
-3', reverse-*5'- CCCTGGGAAAGAACCGAACTG -3'*.

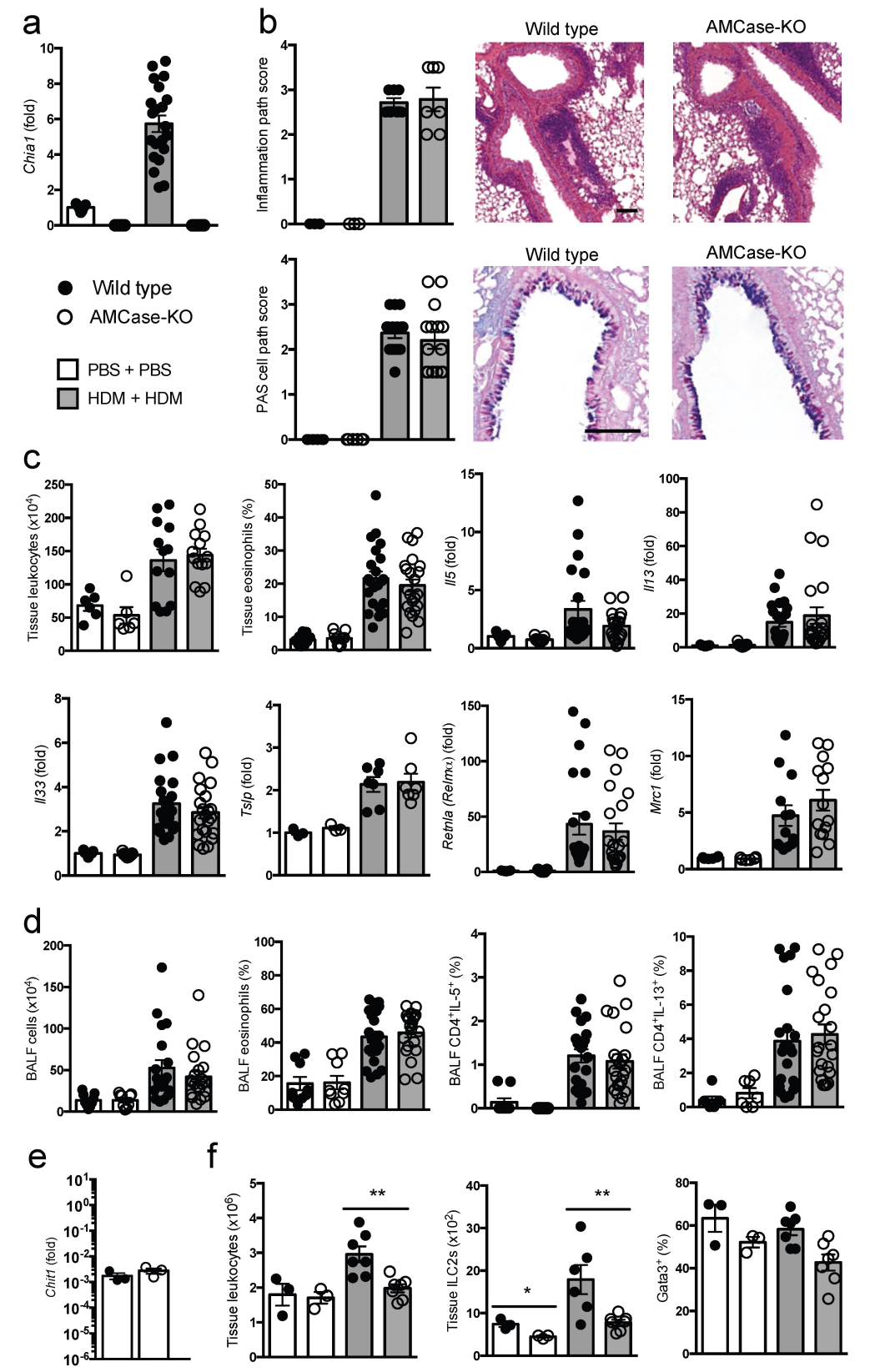
Statistical analysis

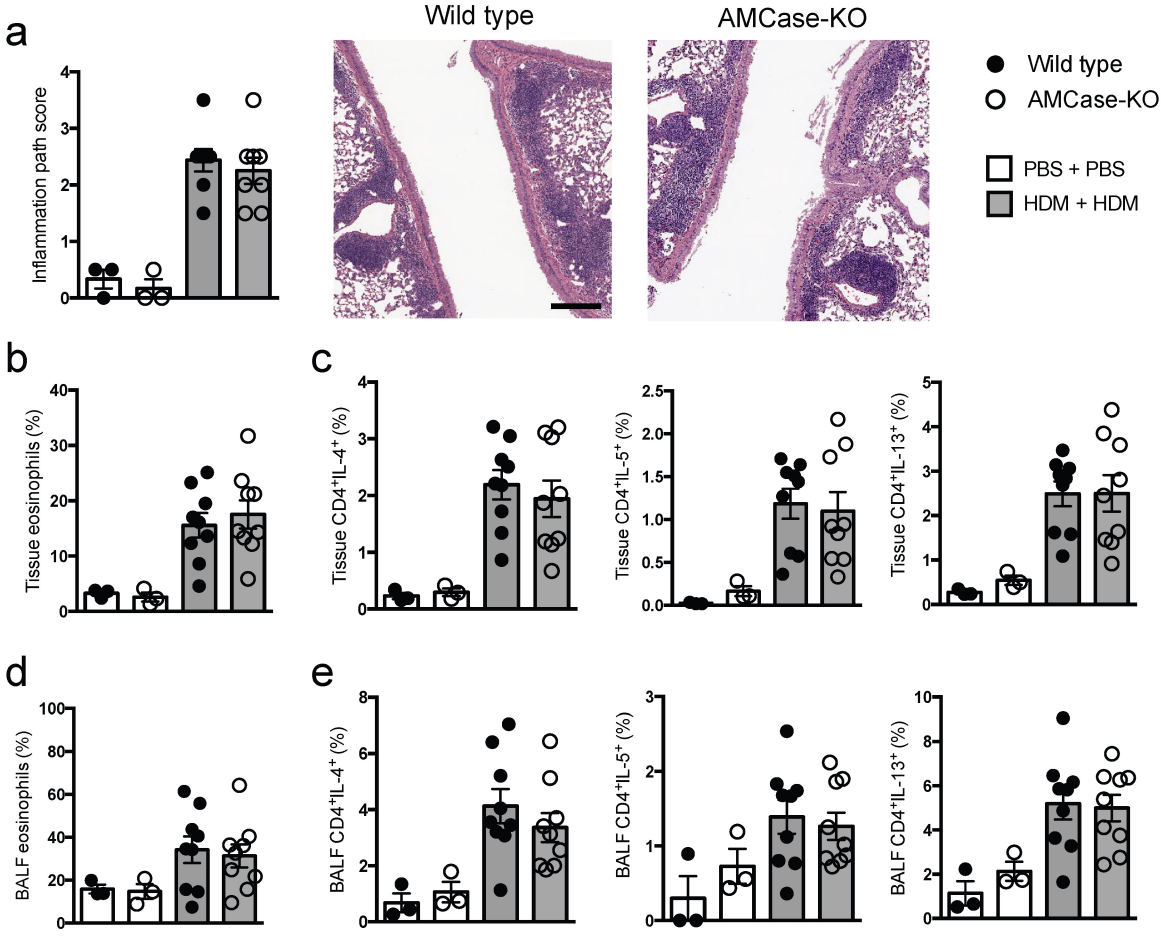
All data were analyzed with Prism (Version 5; GraphPad). Data sets were compared with a two-tailed t-test, and differences were considered significant if *P* values were less than 0.05. No statistical methods were used to predetermine sample size. Group sample size was chosen using records of variance in past experiments, and variance is similar between groups being statistically compared. Mice or samples were randomly assigned to experimental groups or processing orders. Group allocation was blinded for all mouse work, when possible (e.g. administration of allergens and infectious agents, sample quantification and analysis, pathology scoring). Samples or data points were excluded only in the case of a technical equipment or human error that caused a sample to be poorly controlled for.

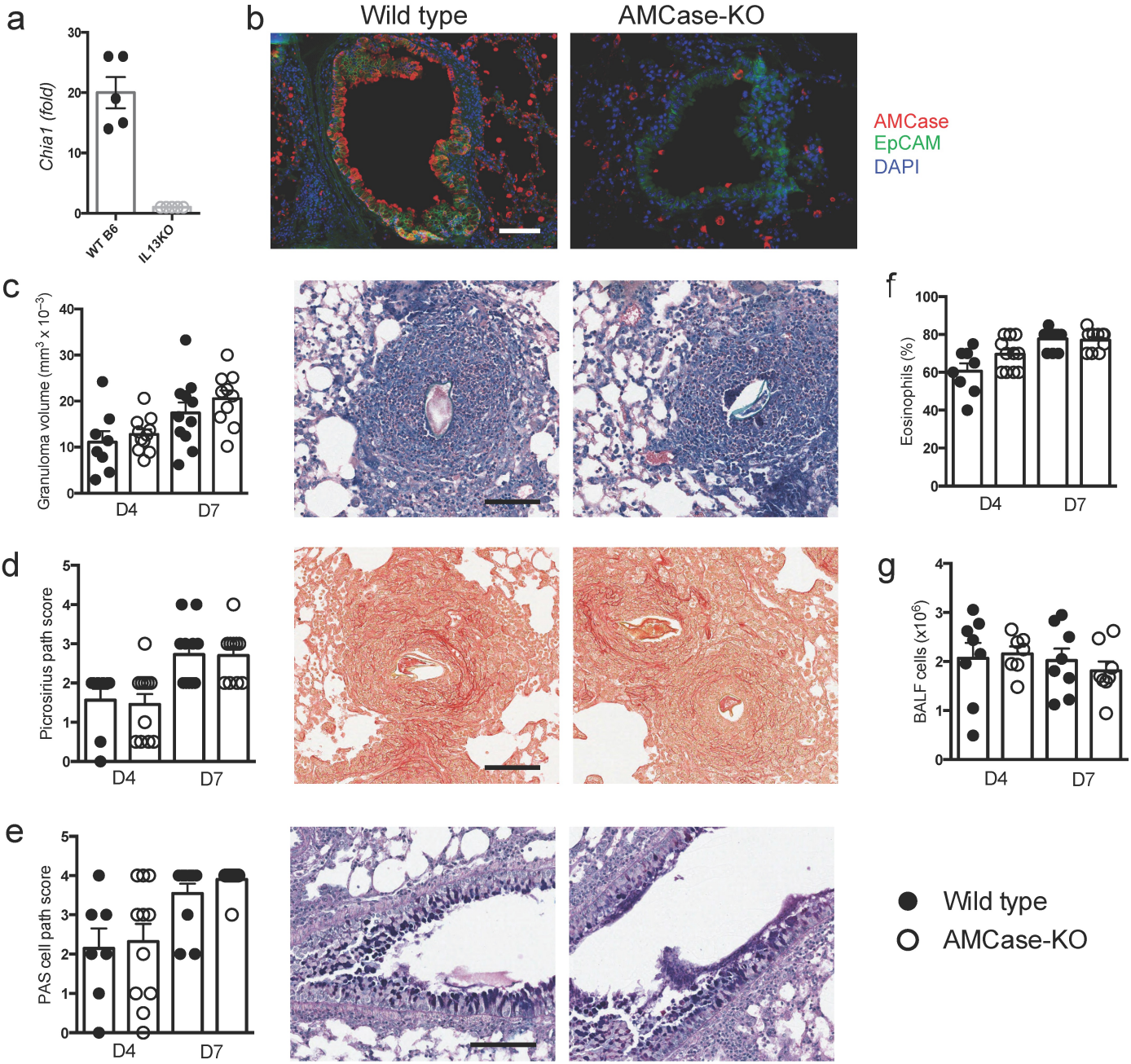
Methods References

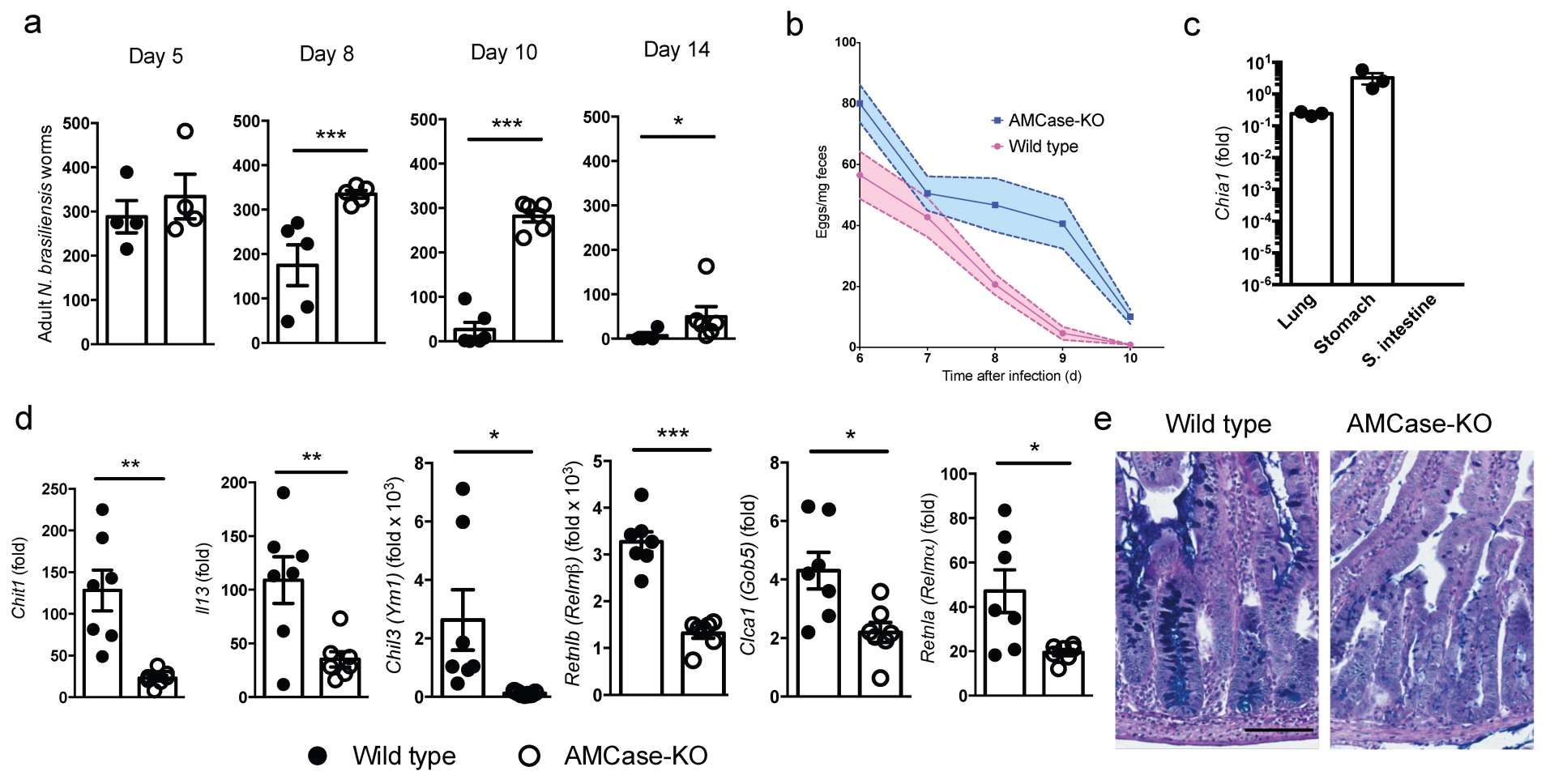
31. Katona, I.M., Urban, J.F., Jr., Scher, I., Kanellopoulos-Langevin, C. & Finkelman, F.D. Induction of an IgE response in mice by Nippostrongylus brasiliensis: characterization of lymphoid cells with intracytoplasmic or surface IgE. *J Immunol* **130**, 350-356 (1983).

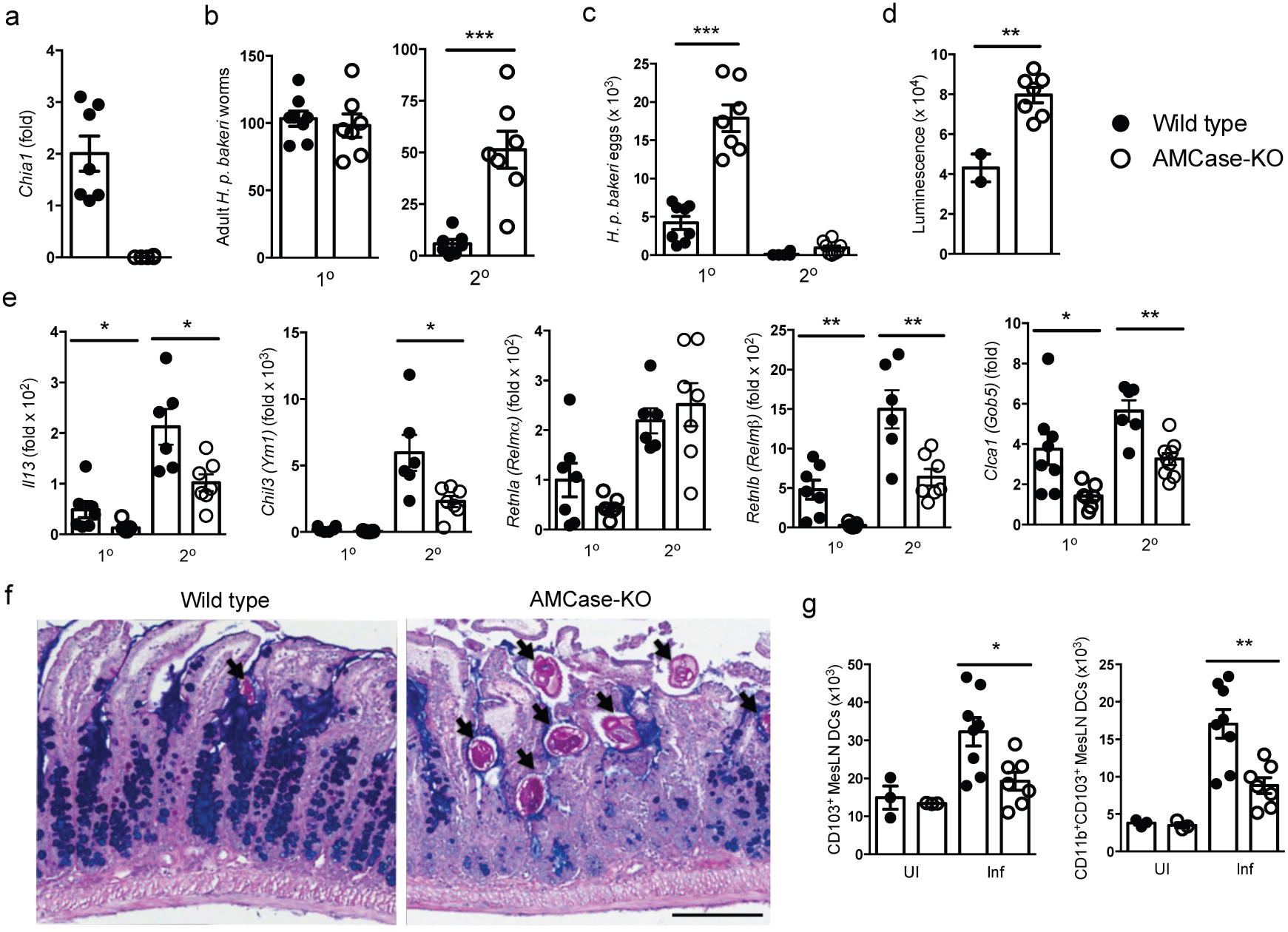
32. Liu, Q. *et al.* B cells have distinct roles in host protection against different nematode parasites. *J Immunol* **184**, 5213-5223 (2010).


a
Chia1 (fold)
Wild type
AMCase-KO
PBS + PBS
HDM + HDM
b
Inflammation path score
Wild type
AMCase-KO
PAS cell path score
Wild type
AMCase-KO
c
Tissue leukocytes (x10⁴)
Tissue eosinophils (%)
Il5 (fold)
Il13 (fold)
Il33 (fold)
Tslp (fold)
Retnla (Relmα) (fold)
Mrc1 (fold)
d
BALF cells (x10⁴)
BALF eosinophils (%)
BALF CD4⁺IL-5⁺ (%)
BALF CD4⁺IL-13⁺ (%)
e
Chit1 (fold)
f
Tissue leukocytes (x10⁶)
**
Tissue ILC2s (x10²)
*
**
Gata3⁺ (%)


a
Wild type
AMCase-KO
Inflammation path score
Wild type
AMCase-KO
PBS + PBS
HDM + HDM
b
Tissue eosinophils (%)
c
Tissue CD4+IL-4+ (%)
Tissue CD4+IL-5+ (%)
Tissue CD4+IL-13+ (%)
d
BALF eosinophils (%)
e
BALF CD4+IL-4+ (%)
BALF CD4+IL-5+ (%)
BALF CD4+IL-13+ (%)


a
Chia1 (fold)
30
20
10
0
WT B6
IL13KO
b
Wild type
AMCase-KO
AMCase
EpCAM
DAPI
c
Granuloma volume (mm3 x 10−3)
40
30
20
10
0
D4
D7
f
Eosinophils (%)
100
80
60
40
20
0
D4
D7
d
Picrosirius path score
5
4
3
2
1
0
D4
D7
g
BALF cells (x10^6)
4
3
2
1
0
D4
D7
e
PAS cell path score
5
4
3
2
1
0
D4
D7
Wild type
AMCase-KO


a
Day 5
Day 8
Day 10
Day 14
Adult N. brasiliensis worms
***
***
*
b
AMCase-KO
Wild type
Eggs/mg feces
Time after infection (d)
c
Chia1 (fold)
Lung
Stomach
S. intestine
d
Chit1 (fold)
Il13 (fold)
Chil3 (Ym1) (fold x 10^3)
Retnlb (Relmβ) (fold x 10^3)
Clca1 (Gob5) (fold)
Retnla (Relmα) (fold)
Wild type
AMCase-KO
e
Wild type
AMCase-KO


a
Chia1 (fold)
b
Adult H. p. bakeri worms
1º
2º
***
c
H. p. bakeri eggs (x 10³)
***
d
Luminescence (x 10⁴)
**
Wild type
AMCase-KO
e
Il13 (fold x 10²)
Chil3 (Ym1) (fold x 10³)
Retnla (Relmα) (fold x 10²)
Retnlb (Relmβ) (fold x 10²)
Clca1 (Gob5) (fold)
f
Wild type
AMCase-KO
g
CD103⁺ MesLN DCs (x10³)
CD11b⁺CD103⁺ MesLN DCs (x10³)
UI
Inf